



HiPur At 4FF

Index

1. Product Description.....	1
2. Purification Procedure.....	2
3. Removal of leached ligand.....	3
4. Cleaning-in-place.....	3
5. Troubleshooting.....	3
6. Related Products.....	3

1. Product Description

Protein At Beads 4FF is an affinity chromatography medium designed for the purification of monoclonal antibody, polyclonal antibody and Fc tag fusion protein. The characteristics of **Protein At Beads 4FF** are summarized in Table 1. Alkali-tolerant protein A (protein At) is obtained by gene recombination technology on the base of natural protein A. **Protein At Beads 4FF** retains the capacity after 200 times repeated CIP cycles with 0.1M NaOH, approximately 80% of the initial capacity with 0.5M NaOH after 100 times repeated CIP cycles, see Figure 1. Protein At is directional immobilized to the agarose beads. The level of leakage of the ligand during elution is very low (less than 10ng ligand/mg antibody, see Figure 2).

The base matrix of **Protein At Beads 4FF** is highly cross-linked agarose derivative with excellent chemical and physical stabilities, making it ideal for process scale applications, see Figure 3.

HiPur At 4FF is a prepacked ready to use column. It is packed with 20 ml **Protein At Beads 4FF**. The arrow on the column label indicates the recommended flow direction. **HiPur At 4FF** can be adapted to all kinds of chromatography system, such as ÄKTA. It is easy to operate.

Table 1. Characteristics of HiPur At 4FF

Item	Description
Size	20 ml
Column size	1.6×10 cm
Matrix Spherical	Highly cross-linked 4% agarose beads
Ligand	Protein At (Alkali-tolerant protein A)
Static Binding Capacity	>40mg human IgG/ml medium
Particle size	45-165um
pH	3-12
Chemical stability	all reagent in the process of antibody purification
Cleaning-in-place	0.1-0.5M NaOH
Velocity	50-300cm/h
Storage	1×PBS containing 20% ethanol, 2℃ - 8℃

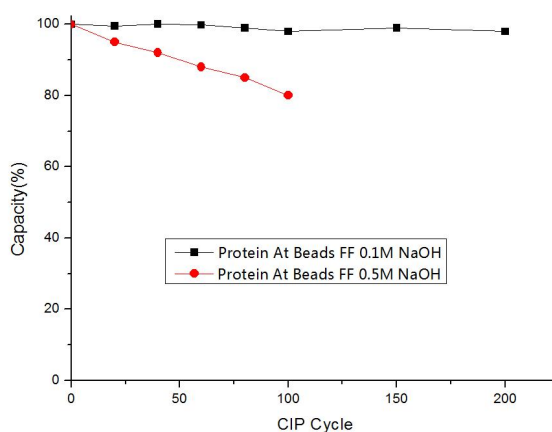


Figure 1. Dynamic binding capacity of Protein At Beads 4FF after CIP with 0.1-0.5M NaOH.



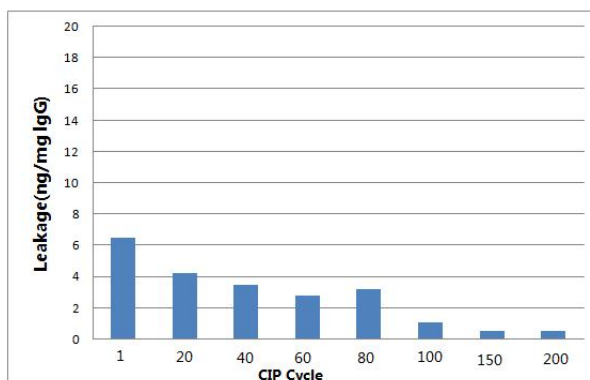


Figure 2. The leakage of the **Protein At Beads 4FF** ligand over numerous CIP cycles.

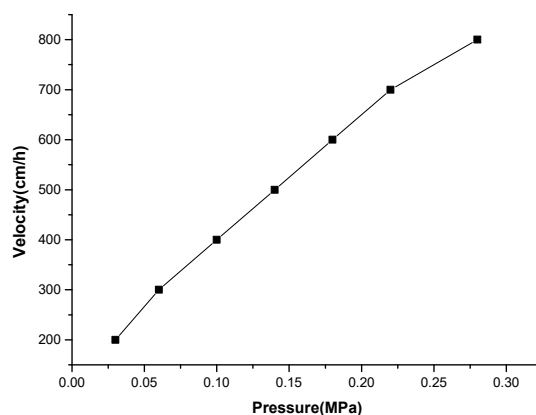


Figure 3. Pressure /flow profile for **Protein At Beads 4FF** in a packed bed (bed height 20cm i.d. 300mm) in BPG300

2. Purification Procedure

2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.22 or 0.45 μm filter before use.

Binding/Wash Buffer: 0.15 M NaCl, 20 mM Na_2HPO_4 , pH 7.0

Elution Buffer: 0.1 M glycine, pH 3.0

Neutralization Buffer: 1 M Tris-HCl, pH 8.5

2.2 Sample Preparation

To insure that proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascite fluid or cell culture supernatant at least 1:1 with binding/wash buffer. Alternatively, the sample may be dialyzed overnight with binding/wash buffer.

2.4 Sample Purification

HiPur At 4FF is a prepacked, ready to use column for purification. The prepacked column provides fast, simple and easy separations in a convenient format.

- 1) Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, "drop to drop" to avoid introducing air into the column. Remove the snap-off end at the column outlet.
- 2) Wash the column with 10 column volumes of binding buffer.
- 3) Load the sample by using a syringe fitted to the connector or by pumping it onto the column.
- 4) Wash the column with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.
- 5) Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.
- 6) Add 10 μl Neutralization Buffer to each 100 μl of eluate to neutralize the pH.

2.5 Analysis

Identify the fractions using UV absorbance, SDS-PAGE, or western blot.





3. Removal of leached ligand

The ligand leakage of **Protein At Beads 4FF** is generally low (less than 10ng ligand/mg antibody). However, in many monoclonal antibody applications it is a requirement to eliminate leached ligand from the final product. There are a number of chromatographic solutions, such as cation exchange chromatography, anion exchange chromatography, or size exclusion chromatography.

4. Cleaning-in-place

In general, **Protein At Beads 4FF** are well suited for reuse a number of times. When precipitation and protein aggregation cause the loss of velocity and combined loads, you need to clean the medium.

Protein At Beads 4FF is a alkali tolerant medium and allows the use of 0.1-0.5 M NaOH for cleaning-in-place(CIP), which improves product quality and reduces overall costs. Each cycle in figure 10 consisted of:

- 3 column volumes binding buffer, pH7.4;
- 0.1 or 0.5 M NaOH, 15min contact time;
- 5 column volumes binding buffer, pH7.4

Note: The viscosity of 0.1–0.5 M NaOH may increase the column pressure. Customers can reverse flushing according to need.

5. Troubleshooting

Problem	Possible Cause	Solution
Back pressure is too high	The filter is blocked.	Clean or replace the filter.
	Column is clogged.	Cleaning in place(part 4).
		Filter the sample solution by passing them through a 0.22µm or 0.45µm filter.
The curve is not stable during sample purification	There are air bubbles in buffer or sample.	De-gas buffers and samples. Do not allow the column to dry.
No antibody in the elute.	The antibody can not be eluted.	Reduce the pH of the elution buffer.
	The antibody is unstable at low pH.	Neutralize the eluted fractions with neutralization buffer immediately after elution.
	The IgG subclass does not bind to protein A.	Try to use the other affinity chromatography media, such as rProtein G Beads, rProtein A/G Beads or PabPur Sulfolink Beads(the specific antigen can be immobilized to the beads).
The recovery rate gradually decreases.	The sample is overloaded.	Reduce the loading volume.
	The reduced performance of the medium.	Cleaning in place(part 4).

6. Related Products

Product	Cat. No.	Size
HiPur At 4FF	SA023C20	20 ml
HiSelect At 4FF	SA023C47	4.7 ml

